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Serial No. 09/922,333

Docket No. D305.001PN.2

**REMARKS****The Claimed Invention**

The claimed invention is directed to xanthene derivative dye compounds that include additional fused rings and precursors useful for synthesizing such dye compounds. The compounds may be present in a form containing a chemical reactive group, or attached to a biological compound or present as an un-reactive dye compound. These dye compounds, in their various forms, are used to stain or label a biological sample wherein the fluorescent compounds provide a detectable signal that identifies the biological sample.

**The Pending Claims**

Prior to entry of the attached amendments, Claims 1-48 are pending. Claims 1-10 are directed to precursor compounds that are useful for synthesizing dihydrodihydroxyquinoline compounds of the invention. Claims 11-14 are directed to dihydrodihydroxyquinoline compounds that are synthesized by the combination of the precursor compounds from Claims 1-10 and an additional precursor. Claims 15-20 are directed to derivatives of xanthene or oxazine compounds synthesized using the precursor compounds from Claims 1-10. Claim 21 is a derivative of seminaphthorhodafluor synthesized using the precursor compounds of Claims 1-10. Claims 22-39 are directed to rhodamine derivatives synthesized using precursor compounds of Claims 1-10. Claims 40-27 are directed to methods for staining a biological sample using compounds in Claims 22-39.

**The Office Action**

Claims 1-48 are restricted.

**Amendments**

Claims 1-22, 24-41, 43-44 and 46-48 have been amended.

Claims 2-10, 12-14, 16-20, 24-39, 41, 43-44 and 46-48 have been amended to replace

'A' with 'The' to indicate dependent form.

Claims 1, 9-11, 13, 15, 20-22, 30-32, 34, 40 and 46 have been amended to clarify the Markush groups.

Claims 1, 11, 15, 21, 22, and 40 have been amended to clarify that the optional alkoxy substituents of R<sup>1</sup>, R<sup>1'</sup>, R<sup>2</sup>, R<sup>2'</sup>, R<sup>6</sup> and R<sup>7</sup> are C<sub>1</sub>-C<sub>6</sub> carbon substituents. Support can be found

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in these claims as filed and in the specification on page 5, line 8; page 6 lines 4-5, 13 and 26; and page 7, line 12.

Claims 1, 11, 15, 21, 22, and 40 have been amended to include 'azido' as an optional substituent of the aryl or heteroaryl moiety of  $R^1$  and  $R^2$ . Support can be found on page 7, lines 15 and 23-24.

Claims 8, 13, 20, and 29 have been amended to replace '1-24 nonhydrogen atoms' with 1-20 non-hydrogen atoms. Support can be found in the claims as filed and on page 28, lines 18-19.

Claims 8, 13, 20, and 29 have been amended to remove the limitation of the type of bonds comprising the linker.

Claims 10, 13, 20 and 32 have been amended to replace 'monosaccharide, a polysaccharide' with 'carbohydrate'. Support can be found on page 30, line 24; page 32, lines 29-31; page 33 lines 1-2 and Table 3.

Claims 11, 15 and 21 have been amended to include  $-L-R_x$  and  $-L-S_c$  as optional substituents when  $R^1$  and  $R^2$  form an aromatic or heteroaromatic ring. Support can be found in Claim 1 as filed and on page 8, lines 15-17.

Claims 11, 15, and 21 have been amended to replace 'aromatic or heteroaromatic' with 'aryl or heteroaryl' in reference to  $R^1$ ,  $R^2$  and  $R^6$ . Support can be found in Claim 1 as filed and on page 6, line 6 and page 7, lines 13-15.

Claim 15 has been amended to clarify that the optional alkoxy substituents of  $R^{20}$  and  $R^{21}$  are  $C_1-C_6$  carbon substituents. Support can be found in these claims as filed and on page 19, line 16.

Claims 15 and 21 have been amended to include  $C_1-C_6$  alkoxy as an optional substituent of the aromatic or heteroaromatic ring of  $R^3$  and  $R^4$ . Support can be found in Claims 1 and 11 as filed and page 6, lines 11-13.

Claims 15, 21, 22 and 40 have been amended to clarify that the alkyl substituents of the alkyl portions of  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are  $C_1-C_6$ , or  $C_2-C_6$  substituents. Support can be found in the claims as filed and page 17, lines 19-23; page 18, lines 1.

Claims 15, 21, 22 and 40 have been amended to replace ' $C_6-C_{18}$ ' of arylcarboxyamido with ' $C_7-C_{18}$ '. Support can be found on page 17, line 19.

Claims 15, 21, 22 and 40 have been amended to clarify that any of the adjacent  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  substituents can be combined to form a fused 6-membered aromatic ring. Support can be found in the claims as filed and on page 18, lines 2-4.

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Claims 15, 21, 22 and 40 have been amended to include  $-L-R_x$  and  $-L-S_c$  as an optional substituent for  $R^{29}$ . Support can be found on page 29, lines 4-5.

Claim 15 has been amended to remove the substituent 'nitro' from the possible list of substituents for  $R^1$ ,  $R^2$  and  $R^6$ .

Claim 15 has been amended to replace 'hydrazine' with 'hydrazino' in regards to  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$ . Support can be found in Claims 21, 22 and 40 as filed and on page 17, lines 16-17.

Claims 21, 22 and 40 have been amended to include  $-L-R_x$  and  $-L-S_c$  as an optional substituent for  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^8$ . Support can be found in claims 1, 11 and 15 as filed and on page 8, lines 14-15; page 29, lines 1-7.

Claims 22 and 40 have been amended to correct a typographical error in the structure as drawn. Support can be found on page 22, lines 15-20.

Claims 22 and 40 have been amended to clarify that the optional alkoxy substituents of  $R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$  and  $R^{46}$  are  $C_1-C_6$  carbon substituents. Support can be found in these claims as filed and on page 5, line 8; page 6, lines 4-5, 13 and 26; page 7, line 12; page 21, lines 15-16 and page 22, lines 19-20.

Claims 22 and 40 have been amended to include  $-L-R_x$  and  $-L-S_c$  as optional substituents when  $R^1$  and  $R^2$  or  $R^{41}$  and  $R^{42}$  form an aromatic or heteroaromatic ring. Support can be found in Claim 1 as filed and on page 8, lines 15-17 and page 22, lines 19-23.

Claims 22 and 40 have been amended to remove the substituent 'nitro' from the list of possible substituents for  $R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$  and  $R^{42}$ .

Claims 22 and 40 have been amended to replace 'aromatic or heteroaromatic' with 'aryl or heteroaryl' in reference to  $R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$  and  $R^{46}$ . Support can be found in Claim 1 as filed and on page 6, line 6; page 7, lines 13-15; page 21, line 17 and page 22, lines 19-20.

Claims 22 and 40 have been amended to include  $C_1-C_6$  alkoxy as an optional substituent of the aromatic or heteroaromatic ring of  $R^3$  and  $R^4$  or  $R^{43}$  and  $R^{44}$ . Support can be found in Claims 1 and 11 as filed and on page 6, lines 11-13 and on page 21, lines 22-25.

Claims 22 and 40 have been amended to include 'sulfonic acid' in regard to the optionally substituted alkyl portions of  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$ . Support can be found in Claim 15 and 21 as filed and page 17, line 23.

Claim 23 has been cancelled without prejudice.

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Claim 28 has been amended to include R<sup>2b</sup> and to remove R groups that are not present in the structure as drawn in Claim 22. Support can be found in Claim 22 as filed and on page 29, line 5.

Claim 30 has been amended to include the reactive groups 'perfluorobenzamido' and 'azidoperfluorobenzamido'. Support can be found in Claim 31 as filed and on page 30, lines 14-15.

Claim 34 and 37 has been amended to include 'lectin' as a Sc. Support can be found in claim 36 as filed and on page 31, line 13 and 16 and Table 3 (page 34).

Claim 40 has been amended to clarify the method staining a sample including the step of combining and the step of illuminating. Support can be found in the claim as filed and on page 35, lines 12-15; page 37, lines 4-11 and page 39, line 31; page 40, lines 1-2.

Claim 40 has been amended to remove the limitation of biological from sample as the compounds of the present invention can label inorganic and organic compounds. Support can be found in the specification on page 25, line 25; page 26, lines 1-3.

Claim 42 has been cancelled without prejudice.

Claim 43 has been amended to include 'growth medium, tissue, proteins, peptides, or biological fluids' as samples. Support can be found on page 36, lines 10, 14, 19 and page 38, lines 3-9.

Claim 45 has been cancelled without prejudice.

Claims 46 and 48 have been amended to remove R groups that are not present in the structure as drawn in Claim 22.

Support for new Claims 49-53 is found in the claims as filed and throughout the specification, and in particular the limitations associated with the compounds of claims 49-53 can be found in Claims 22-49 and on page 40, lines 18-28 and page 21, lines 8-26; page 22, lines 1-20.

Applicants believe that no new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

**RESPONSE TO THE RESTRICTION REQUIREMENT**

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In the response that follows, the Examiner's Election/Restriction of the Applicant's claimed invention is provided in full text, as identified by indented small bold print, followed by the Applicants response.

**35 U.S.C. 121 Restriction**

- I. **Claims 1 to 10, drawn to compounds of the formula shown in claim 1, classified in class 546, subclass 79+.**
- II. **Claims 11 to 14, drawn to compounds of the formula shown in claim 11, classified in class 546, subclass 61+.**
- III. **Claims 15 to 20, drawn to compounds of the formula shown in claim 15, classified in class 544, subclass 89+.**
- IV. **Claim 21, drawn to compounds of the formula shown in claim 21, classified in classes 544 and 546, various subclasses.**
- V. **Claims 22 to 39, drawn to compounds of the formula shown in claim 22, classified in class 546, subclass 33+.**
- VI. **Claims 40 to 48, drawn to a method of staining a biological sample, classified in class 436, subclass 172+.**

Applicants respectfully traverse the above restriction requirement and request reconsideration. As required by CFR 1.143, Applicants provisionally elect Group V as drawn to compounds of the formula shown in Claim 22.

**The compounds of groups I to V are independent and distinct from one another because of their structural differences. The compounds contain a different number of fused rings.**

Applicants traverse the restriction requirement and respectfully request that the Examiner reconsider rejoining the compound claims (Groups I-V). Group I represents compounds that are directed to intermediate compounds that are used to synthesize the compounds of Groups II, III, IV and V. As such, the compounds of these groups are thus related by the presence of Group I compounds. The multiple fused rings of the compounds in group I confer novelty, when present alone (Group I), or individually as part of a larger compound structure (Groups II, III and IV), or as part of a symmetrical compound (Group V). Therefore, the compounds of Group I are present within all of the compounds of the present invention. Therefore, applicants respectfully assert that a search based on the fused rings of Group I compounds will encompass all of the compounds of the present invention.

While the compounds of the present invention (Groups I-V) may contain a different number of fused rings, the point of novelty remains constant with three to six fused rings of the

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Group I compounds. Applicants respectfully assert that the compounds of Groups I-V are not patentably distinct and should be rejoined as all the compounds comprise the fused rings of the Group I compounds.

Inventions V and VI are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product claimed can be used in a materially different process of using the product (MPEP § 806.05(h)). In the instant case the compounds of group V can be used to dye textile materials.

The applicants respectfully request that the method claims of Group VI be re-grouped with the compound claims of Group V. The method of Group VI is directed to staining of a sample and applicants respectfully assert that the method of dyeing textile material falls within the scope of these claims. Textile materials include biological materials such as wool and cotton or non-biological material such as nylon comprising synthetic polymers, and when presented on a microscopic level they fall within the definition of "sample" provided by the Applicants.

Staining of a sample can be accomplished by one of three routes, depending on the compounds selected to stain. First (1), when the compounds contain a reactive group the compounds can form a covalent bond with another substance that contains a reactive group, thus, any conjugated substance is also considered within the definition of a sample. Second (2), when the dye compound is covalently attached to a specific binding partner that has affinity for another substance, such as an antibody and an antigen, then the antibody or antigen or both are considered within the definition of a sample. Third (3), when the dye compound does not comprise a reactive group or a conjugated substance, the dye compounds stain a sample when present within the bounds of the sample, which includes textile materials.

The first (1) route comprises a compound of the present invention with a reactive group that will form a covalent bond with another substance that also comprises a reactive group such that the reactive dye compound effectively labels or stains the substance with a fluorescent dye compound. The specification states that "the dyes with a reactive group ( $R_x$ ) fluorescently label a wide variety of organic and inorganic substances that contain or are modified to contain functional groups with suitable reactivity, resulting in chemical attachment of the conjugated substance ( $S_o$ ), represented by  $-L-S_o$ " (p. 25 line 25; p. 26 lines 1-3). Examples of some

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conjugated substances are provided on page 30 lines 21-24 and include "antigens, steroids, vitamins, drugs, haptens, metabolites, toxins, environmental pollutants, amino acids, peptides, proteins, nucleic acids, nucleic acid polymers, carbohydrates, lipids, ion-complexing moieties, and non-biological polymers". Based on the biological and non-biological polymers that make up textile materials, these materials fall within the definition of the preferred conjugated substances provided in the specification.

The second (2) route comprises dye compounds of the present invention that contain a conjugated substance as defined in the specification wherein another substance may be labeled or stained when it specifically interacts with the conjugated substance (Page 34, lines 18-27 and Table 3). This is exemplified with a conjugated substance of an antibody and an antigen, wherein antigen is defined as any substance that elicits a response in an animal to produce antibodies that have affinity for the antigen. The antigen is typically a biological substance but inorganic non-biological substances, such as latex, also function as antigens. Therefore, textile materials such as wool (keratin), cotton or the synthetic polymers that comprise nylon may be labeled using the compounds of the present invention when present as a conjugated substance. Therefore, such methods fall within the scope of the claims in Group IV.

The third (3) route comprises compounds that do not contain a reactive group ( $R_x$ ) or a conjugated substance ( $S_c$ ), the compounds of the present invention can stain a sample when present within the bounds of the sample without the formation of a covalent or non-covalent bond. The specification does not exclude textile materials and includes them on a microscopic level by the inclusion of amino acid polymers (wool and cotton) and non-biological polymers (nylon). The specification provides example of polymers, includes amino acids such as peptides and proteins (page 31, line 9), nucleic acid polymers such as natural or synthetic DNA and RNA (page 31, lines 29-30), carbohydrates such as polysaccharides including agarose and dextran (page 32 lines 29-31) and other organic and inorganic polymers including "polymeric films, polymeric wafers, polymeric membranes, polymeric particles, or polymeric microparticles, including magnetic and non-magnetic microspheres, conducting and non-conducting metals and non-metals, and glass and plastic surfaces and particles" (page 33, lines 29-31). Thus, staining of textile materials falls within the scope of Claims 40-49 for staining a sample.

In summary, when a compound of the present invention, present in various forms as described above, is added to a sample that sample is effectively labeled or stained with a fluorescent dye. The sample includes organic and inorganic substances that when present on a

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microscopic level cover the methods of labeling textile materials using the compounds of the present invention.

### CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 984-5656.

Respectfully submitted,

Date: October 10, 2002

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Diwu *et al.*

Serial No.: 09/922,333

Filed: August 4, 2001

For: **DERIVATIVES OF 1,2-DIHYDRO-7-HYDROXYQUINOLINES CONTAINING FUSED RINGS**

Examiner: F. Powers

Group Art Unit: 1626

**MARKED-UP VERSION OF THE CLAIMS**

Assistant Commissioner for Patents  
U.S. Patent and Trademark Office  
Washington, D.C. 20231

Dear Sir:

The following Marked-up Version of the Claims is hereby submitted together with a Clean Version of the Claims and the Response to Restriction Requirement on or before the due date of October 10, 2002.

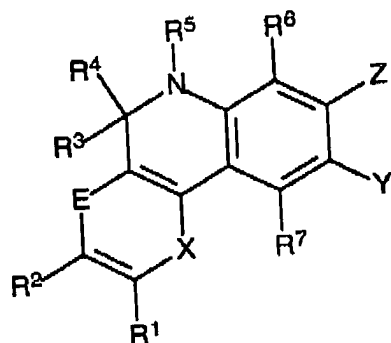
**CERTIFICATE OF TRANSMISSION**

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HERewith ARE BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADEMARK OFFICE ON \_\_\_\_\_ TO  
1.703.872.9306 By \_\_\_\_\_

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1. (Amended) A compound [of the] comprising a formula



wherein

$R^1$  and  $R^2$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or [a]  $C_1$ - $C_6$  alkyl, [or]  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl, - $L$ - $R_x$  and - $L$ - $S_c$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring] and said aryl or heteroaryl [that] is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or - $L$ - $R_x$ ; or - $L$ - $S_c$ ]

or  $R^1$  in combination with  $R^2$  forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or [which] said ring is substituted by - $L$ - $R_x$  or - $L$ - $S_c$ ;

or  $R^2$  in combination with  $R^3$  forms a 5- or 6-membered alicyclic ring;

$R^3$  and  $R^4$  are independently selected from the group consisting of [H] hydrogen,  $C_1$ - $C_6$  alkyl, aromatic or heteroaromatic ring, - $L$ - $R_x$  and - $L$ - $S_c$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or

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an aromatic or heteroaromatic ring] and said aromatic or heteroaromatic ring [that] is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>]

or R<sup>3</sup> in combination with R<sup>4</sup> forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [a] C<sub>2</sub>-C<sub>6</sub> alkyl, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that] and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>]

R<sup>6</sup> is independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or a] C<sub>1</sub>-C<sub>6</sub> alkyl, [or] C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an] and said aryl or heteroaryl [ring that] is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>]

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

R<sup>7</sup> is independently selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl [having 1-6 carbons, or] C<sub>1</sub>-C<sub>6</sub> alkoxy [having 1-6 carbons; or] -L-R<sub>x</sub>; or] and -L-S<sub>c</sub>;

one of X and E is O, S, NR<sup>8</sup>, or CR<sup>1</sup>'=CR<sup>2</sup>', and the other is absent;

wherein R<sup>8</sup> is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [or a] C<sub>2</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl

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[that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen;  
[or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;] and

R<sup>1</sup> and R<sup>2</sup> are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or [a] C<sub>1</sub>-C<sub>6</sub> alkyl, [or] C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring] and said aryl or heteroaryl [that] is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;]

Y is independently selected from the group consisting of H, OH, NH<sub>2</sub>, NO, -(CO)-R<sup>9</sup>, -(CO)-O-R<sup>10</sup>, wherein said R<sup>9</sup> and R<sup>10</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl, or a substituted or unsubstituted aryl or heteroaryl ring system having 1-2 rings;

Z is independently selected from the group consisting of H, OH, NHR<sup>17</sup>, SH, or C(CR<sup>11</sup>R<sup>12</sup>)<sub>2</sub>OH; wherein said R<sup>17</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen;] and said R<sup>11</sup> and R<sup>12</sup> are independently C<sub>1</sub>-C<sub>6</sub> alkyl that are optionally substituted by carboxylic acid, sulfonic acid, or halogen, or R<sup>11</sup> and R<sup>12</sup> taken in combination form a 5- or 6-membered alicyclic ring;

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

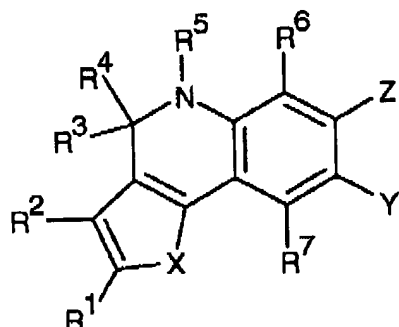
S<sub>c</sub> is a conjugated substance.

2. (Amended) [A] The compound, as claimed in] according to Claim 1, wherein one of X and E is O, S, or CR<sup>1</sup>=CR<sup>2</sup>, and the other is absent.

3. (Amended) [A] The compound, as claimed in] according to Claim [1] 2, wherein said compound [having the] has the formula

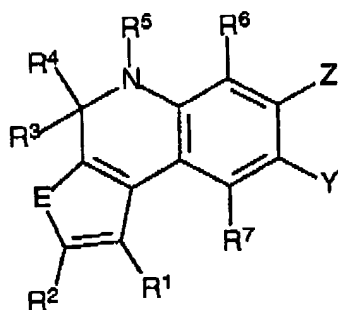
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wherein X is O or S.

4. (Amended) [A] The compound[, as claimed in] according to Claim [1] 2, wherein said compound [having] has the formula



wherein E is O or S.

5. (Amended) [A] The compound[, as claimed in] according to Claim [2] 3, wherein X is S.

6. (Amended) [A] The compound[, as claimed in] according to Claim 1, wherein

R<sup>1</sup> is [H] hydrogen or sulfonic acid;

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R<sup>3</sup> and R<sup>4</sup> are each methyl;

R<sup>6</sup> and R<sup>7</sup> are each hydrogen or methyl; and

Z is OH.

7. (Amended) [A] The compound[, as claimed in] according to Claim 1, wherein Y is H or -(CO)-H or NO.

8. (Amended) [A] The compound[, as claimed in] according to Claim 1, wherein [each] said L is independently a single covalent bond[,] or [L is] a covalent linkage having 1-2[4]0 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S, [and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds.]

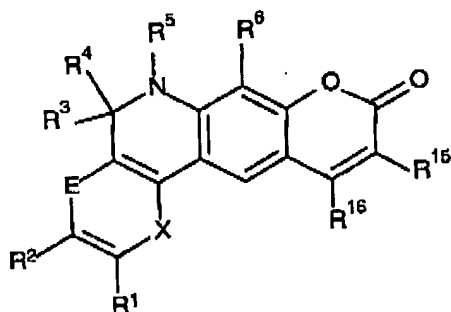
9. (Amended) [A] The compound[, as claimed in] according to Claim 1, wherein said Rx is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, [or] and a thiol group.

10. (Amended) [A] The compound[, as claimed in] according to Claim 1, wherein said Sc is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a [monosaccharide, a polysaccharide] carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, [or] and a virus.

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11. (Amended) A compound [of the] comprising a formula



wherein  $R^1$ ,  $R^2$ , and  $R^6$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or a  $C_1$ - $C_6$  alkyl, [or]  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl, - $L$ - $R_x$  and - $L$ - $S_c$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that] and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or - $L$ - $R_x$ ; or - $L$ - $S_c$ ]

or  $R^1$  in combination with  $R^2$  forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by - $L$ - $R_x$  or - $L$ - $S_c$ ;

$R^3$  and  $R^4$  are independently selected from the group consisting of [H] hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic or heteroaromatic ring, - $L$ - $R_x$  and - $L$ - $S_c$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or] and said [an] aromatic or heteroaromatic ring [that] is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or - $L$ - $R_x$ ; or - $L$ - $S_c$ ]

or  $R^2$  in combination with  $R^3$ , or  $R^3$  in combination with  $R^4$ , forms a 5- or 6-membered alicyclic ring;

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$R^5$  is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [a]  $C_2-C_6$  alkyl, aryl, heteroaryl,  $-L-R_X$  and  $-L-S_C$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen[; or  $R^5$  is an] and said aryl or heteroaryl [ring that] is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or  $-L-R_X$ ; or  $-L-S_C$ ]

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , forms a 5- or 6-membered alicyclic ring;

one of X and E is O, S,  $NR^8$ , or  $CR^{1'}=CR^{2'}[;]$  and the other is absent;

wherein  $R^8$  is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [or a]  $C_2-C_6$  alkyl,  $-L-R_X$  and  $-L-S_C$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; [or  $-L-R_X$ ; or  $-L-S_C$ ] and

$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid[; or] , [a]  $C_1-C_6$  alkyl, [or]  $C_1-C_6$  alkoxy, aryl, heteroaryl,  $-L-R_X$  and  $-L-S_C$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen[; or an aryl or heteroaryl ring] and said aryl or heteroaryl [that] is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or  $-L-R_X$ ; or  $-L-S_C$ ]

$R^{15}$  and  $R^{16}$  are independently selected from the group consisting of hydrogen, cyano, nitro, halogen, carboxylic acid, [or] sulfonic acid[; or a]  $C_1-C_6$  alkyl, an aromatic or heteroaromatic ring system having 1-2 fused rings,  $-L-R_X$  and  $-L-S_C$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen[; or an] and said aromatic or heteroaromatic ring system [having 1-2 fused rings that] is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or  $-L-R_X$ ; or  $-L-S_C$ ]



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wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance.

12. (Amended) [A] The compound[, as claimed in] according to Claim 11, wherein said one of X and E is O or S.

13. (Amended) [A] The compound[, as claimed in] according to Claim 12, wherein

R<sup>6</sup> and R<sup>7</sup> are [H] hydrogen;

R<sup>3</sup> and R<sup>4</sup> are each methyl;

R<sup>1</sup> is [H] hydrogen or sulfonic acid;

one of R<sup>15</sup> and R<sup>16</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>, and the other is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl; or cyano;

wherein L is a single covalent bond, or L is a covalent linkage having 1-2[4]0 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S [and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds], and wherein R<sub>x</sub>[, when present,] is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide,

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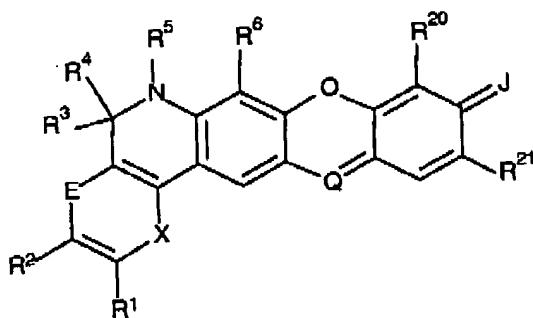
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a phosphoramidite, a reactive platinum complex, a sulfonyl halide, [or] and a thiol group;  
and

wherein  $S_C$  [, when present,] is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a [monosaccharide, a polysaccharide] carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, [or] and a virus.

14. (Amended) [A] The compound[, as claimed in] according to Claim 11, wherein one of said  $R^{15}$  [and] or  $R^{16}$  is an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl.

15. A compound [of the] comprising a formula:



[wherein]

wherein  $R^1$ ,  $R^2$ , and  $R^6$  are independently selected from the group consisting of [H] hydrogen, cyano, [nitro,] halogen, carboxylic acid, [or] sulfonic acid; or a  $C_1$ - $C_6$  alkyl, [or]  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl, -L- $R_x$  and -L- $S_C$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that] and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or -L- $R_x$ ; or -L- $S_C$ ]

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or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R<sub>x</sub> or -L-S<sub>c</sub>;

R<sup>3</sup> and R<sup>4</sup> are independently selected from the group consisting of [H] hydrogen, C<sub>1</sub>-C<sub>8</sub> alkyl, an aromatic or heteroaromatic ring, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen[; or] and said [an] aromatic or heteroaromatic ring [that] is optionally substituted one or more times by C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>1</sub>-C<sub>8</sub> alkoxy, C<sub>1</sub>-C<sub>8</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>]

or R<sup>2</sup> in combination with R<sup>3</sup>, or R<sup>3</sup> in combination with R<sup>4</sup>, forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [a] C<sub>2</sub>-C<sub>6</sub> alkyl, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen[; or R<sup>5</sup> is an] and said aryl or heteroaryl [ring that] is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>]

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

one of X and E is O, S, NR<sup>6</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>[;], and the other is absent;

wherein R<sup>6</sup> is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [or a] C<sub>2</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>] and

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$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or ], [a]  $C_1-C_6$  alkyl, [or]  $C_1-C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring] and said aryl or heteroaryl [that] is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or  $-L-R_x$ ; or  $-L-S_c$ ]

$R^{20}$  and  $R^{21}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or a],  $C_1-C_6$  alkyl [or],  $C_1-C_6$  alkoxy, aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an] said aromatic or heteroaromatic ring [that] is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or  $-L-R_x$ ; or  $-L-S_c$ ]

J is O or  $NR^{37}R^{38}$ ;

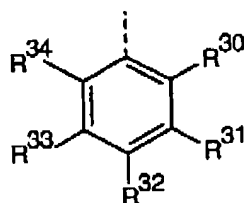
wherein  $R^{37}$  and  $R^{38}$  are independently selected from the group consisting of [H] hydrogen,  $C_1-C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; an aryl or heteroaryl ring]; or  $R^{37}$  in combination with  $R^{38}$  forms a saturated 5- or 6-membered heterocycle that is a piperidine, a morpholine, a pyrrolidine or a piperazine, wherein said heterocycle is [each of which is] optionally substituted by methyl, carboxylic acid, or a carboxylic acid ester of a  $C_1-C_6$  alkyl; [or  $-L-R_x$  or  $-L-S_c$ ]

or  $R^{37}$  in combination with  $R^{20}$ , or  $R^{38}$  in combination with  $R^{21}$ , or both, form a 5- or 6-membered ring that is saturated or unsaturated, and is optionally substituted by one or more sulfonic acids, or  $C_1-C_6$  alkyl that is optionally substituted by sulfonic acid;

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Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is independently selected from the group consisting of [H] hydrogen, F, CN, carboxylic acid, [or] a carboxylic acid ester of a C<sub>1</sub>-C<sub>8</sub> alcohol; or R<sup>28</sup> is] a C<sub>1</sub>-C<sub>8</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>C</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> [has the] comprises a formula



wherein R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently selected from the group consisting of [H] hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, [hydrazine; or] hydrazino, C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, [or C<sub>6</sub>-C<sub>18</sub>] C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>C</sub>, wherein said [the] alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> [which] are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>8</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>8</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino [or] and C<sub>1</sub>-C<sub>8</sub> alkoxy, the alkyl portions of each having 1-6 carbons]; or [one] a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents [R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>,] when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; [or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>C</sub>]; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>C</sub> is a conjugated substance.

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16. (Amended) [A] The compound[, as claimed in] according to Claim 15, wherein said Q is N.

17. (Amended) [A] The compound[, as claimed in] according to Claim 15, wherein said J is O and said Q is CR<sup>28</sup>.

18. (Amended) [A] The compound[, as claimed in] according to Claim 17, wherein one of said R<sup>5</sup>, R<sup>21</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, and R<sup>34</sup> is -L-R<sub>x</sub> or -L-Sc.

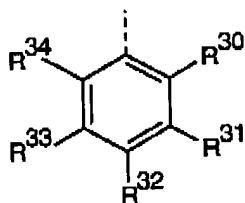
19. (Amended) [A] The compound[, as claimed in] according to Claim 15, wherein said R<sup>3</sup> and R<sup>4</sup> are each methyl;

R<sup>1</sup> is H or a sulfonic acid;

R<sup>6</sup> is H; and

J is NR<sup>37</sup>R<sup>38</sup>.

20. (Amended) [A] The compound[, as claimed in] according to Claim 19, wherein Q [has the formula] is CR<sup>28</sup>[, wherein] and R<sup>28</sup> has the formula



wherein one of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, and R<sup>34</sup> is -L-R<sub>x</sub> or -L-Sc; and  
wherein L is a single covalent bond, or L is a covalent linkage having 1-2[4]0  
nonhydrogen atoms selected from the group consisting of C, N, O, P, and S [and is  
composed of any combination of single, double, triple or aromatic carbon-carbon bonds,

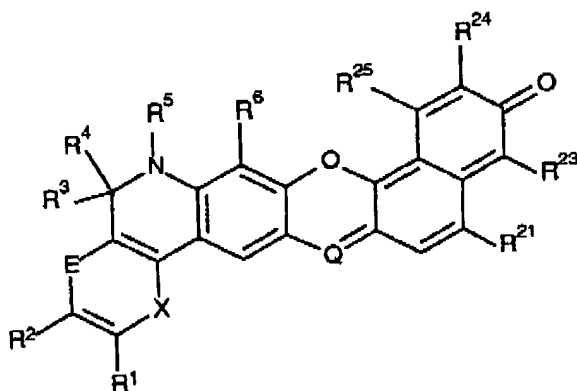
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carbon–nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds], and wherein  $R_x$  [, when present,] is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, [or] and a thiol group; and

wherein  $S_c$  [, when present,] is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a [monosaccharide, a polysaccharide] carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, [or] and a virus.

21. (Amended) A compound [of the] comprising a formula



[wherein]

wherein  $R^1$ ,  $R^2$ , and  $R^6$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or a  $C_1$ - $C_6$  alkyl, [or]  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl, - $L$ - $R_x$  and - $L$ - $S_c$ , wherein said alkyl or alkoxy [that] is optionally

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substituted by carboxylic acid, sulfonic acid, or halogen[; or an aromatic or heteroaromatic ring that] and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or -L-R<sub>x</sub>; or -L-S<sub>c</sub>]

or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R<sub>x</sub> or -L-S<sub>c</sub>;

R<sup>3</sup> and R<sup>4</sup> are independently selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, an aromatic or heteroaromatic ring, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen[; or] and said [an] aromatic or heteroaromatic ring [that] is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>2</sup> in combination with R<sup>3</sup>, or R<sup>3</sup> in combination with R<sup>4</sup>, forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [a] C<sub>2</sub>-C<sub>6</sub> alkyl, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen[; or R<sup>5</sup> is an] and said aryl or heteroaryl [ring that] is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

one of E and X is O, S, NR<sup>a</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>[;], and the other is absent;

wherein R<sup>a</sup> is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [or a] C<sub>2</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl



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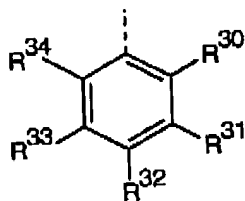
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[that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen;  
and

$R^1$  and  $R^2$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid; or  $\text{--} [a] \text{C}_1\text{--C}_6 \text{ alkyl, [or] C}_1\text{--C}_6 \text{ alkoxy, aryl, heteroaryl, --L-R}_x \text{ and --L-S}_c \text{, wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring] and said aryl or heteroaryl [that] is optionally substituted one or more times by C}_1\text{--C}_6 \text{ alkyl, C}_1\text{--C}_6 \text{ perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;}$

$R^{21}$ ,  $R^{23}$ ,  $R^{24}$ , and  $R^{25}$  are independently selected from the group consisting of hydrogen, cyano, nitro, halogen, carboxylic acid, [or] sulfonic acid; or  $\text{a C}_1\text{--C}_6 \text{ alkyl [or, aromatic or heteroaromatic ring, --L-R}_x \text{ and --L-S}_c \text{, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an said aromatic or heteroaromatic ring [that] is optionally substituted one or more times by C}_1\text{--C}_6 \text{ alkyl, C}_1\text{--C}_6 \text{ perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; [or --L-R}_x \text{ or --L-S}_c \text{;]}$

Q is N or  $\text{CR}^{28}$ , wherein  $R^{28}$  is independently selected from the group consisting of [H] hydrogen, F, CN, carboxylic acid, [or] a carboxylic acid ester of a C}\_1\text{--C}\_6 \text{ alcohol; or } R^{28} \text{ is } [a] \text{ C}\_1\text{--C}\_6 \text{ alkyl, --L-R}\_x \text{ and --L-S}\_c \text{, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or } R^{28} \text{ [has the] comprises a formula}



wherein  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are independently selected from the group consisting of [H] hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or  $\text{C}_1\text{--C}_{18} \text{ alkyl, C}_1\text{--C}_{18} \text{ alkoxy, C}_1\text{--C}_{18} \text{ alkylthio, C}_1\text{--C}_{18}$

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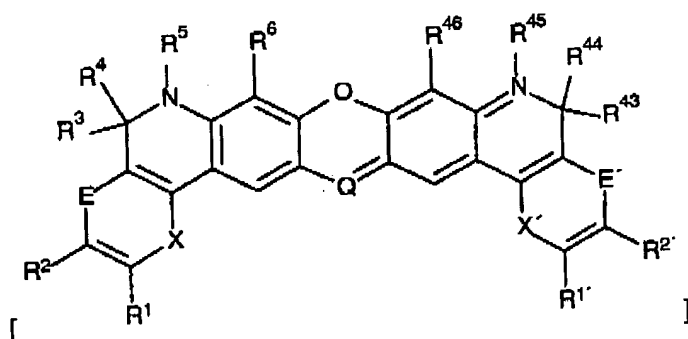
alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkylloxycarbonyl, [or C<sub>6</sub>-C<sub>18</sub>] C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>C</sub>, wherein said [the] alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> [which] are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>8</sub> alkylamino, C<sub>2</sub>-C<sub>8</sub> dialkylamino [or] and C<sub>1</sub>-C<sub>8</sub> alkoxy[, the alkyl portions of each having 1-6 carbons]; or [one] a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents [R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>,] when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; [or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>C</sub>.] and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

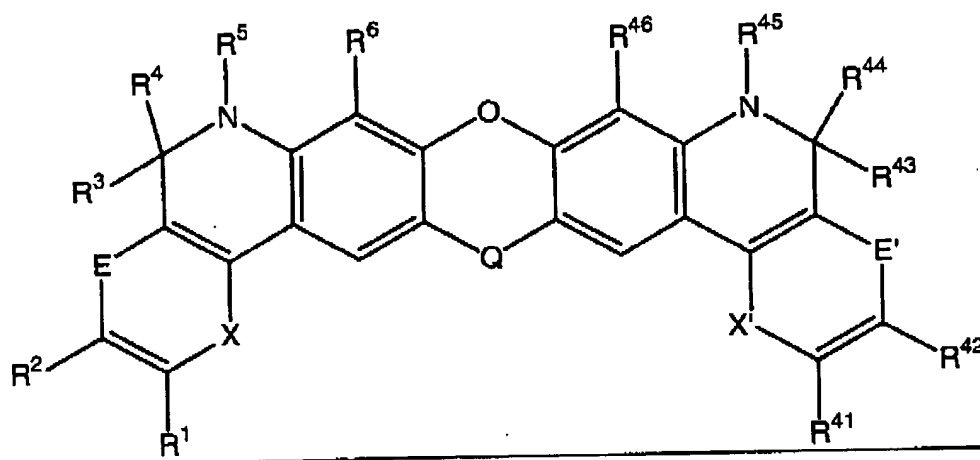
S<sub>C</sub> is a conjugated substance.

22. (Amended) A compound [of the] comprising a formula:



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[wherein]

wherein  $R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$ , and  $R^{46}$  are independently selected from the group consisting of [H] hydrogen, cyano, [nitro,] halogen, carboxylic acid, [or] sulfonic acid; or a)  $C_1$ - $C_6$  alkyl, [or]  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that] and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or  $-L-R_x$  or  $-L-S_c$ ]

or  $R^1$  in combination with  $R^2$ , or  $R^{41}$  in combination with  $R^{42}$ , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by  $-L-R_x$  or  $-L-S_c$ ;

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently selected from the group consisting of [H] hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or] and said [an] aromatic or heteroaromatic ring [that] is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

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or  $R^2$  in combination with  $R^3$ , or  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [a]  $C_2$ - $C_8$  alkyl, aryl, heteroaryl, -L- $R_X$  and -L- $S_C$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen[; or  $R^5$  is an] and said aryl or heteroaryl [ring that] is optionally substituted one or more times by  $C_1$ - $C_8$  alkyl,  $C_1$ - $C_8$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E [and],  $E'$ ,  $X'$  and X is O, S,  $NR^8$ , or  $CR^1=CR^2$ [; the other is absent; and one of  $E'$  and  $X'$  is O, S,  $NR^8$ , or  $CR^1=CR^2$ ; the other is absent;] provided that E and X or  $E'$  and  $X'$  are not both present;

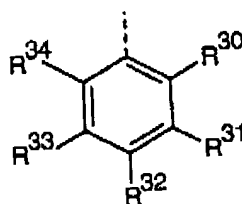
wherein  $R^8$  is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [or a]  $C_2$ - $C_8$  alkyl, -L- $R_X$  and -L- $S_C$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid[; or]  $\Delta$  [a]  $C_1$ - $C_8$  alkyl, [or]  $C_1$ - $C_8$  alkoxy, aryl, heteroaryl, -L- $R_X$  and -L- $S_C$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen[; or an aryl or heteroaryl ring] and said aryl or heteroaryl [that] is optionally substituted one or more times by  $C_1$ - $C_8$  alkyl,  $C_1$ - $C_8$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

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Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is independently selected from the group consisting of [H] hydrogen, F, CN, carboxylic acid, [or] a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is] a C<sub>1</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> [has the] comprises a formula



wherein R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently selected from the group consisting of [H] hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or] C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkylloxycarbonyl, [or C<sub>6</sub>-C<sub>18</sub>] C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said [the] alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> [which] are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino [or] and C<sub>1</sub>-C<sub>6</sub> alkoxy, the alkyl portions of each having 1-6 carbons]; or [one] a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents [R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>,] when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; [or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>] and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance.

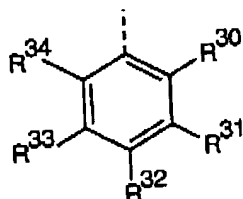
23. (Cancel) A compound, as claimed in Claim 22, wherein

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$X = X'$ ,  $E = E'$ ,  $R^1 = R^{41}$ , and  $R^2 = R^{42}$ .

24. (Amended) [A] The compound[, as claimed in] according to Claim 22, wherein Q [has the formula] is  $CR^{28}$ [, wherein] and  $R^{28}$  has the formula



25. (Amended) [A] The compound[, as claimed in] according to Claim 24, wherein one of  $R^5$ ,  $[R^{21}]$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ , and  $R^{45}$  is  $-L-R_x$  or  $-L-Sc$ .

26. (Amended) [A] The compound[, as claimed in] according to Claim 24, wherein

said  $R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are each methyl;

each  $R^1$  and  $R^{41}$  [are] is independently H or sulfonic acid; and

$R^6$  and  $R^{46}$  are H.

27. (Amended) [A] The compound[, as claimed in] according to Claim 24, wherein [the] said compound is substituted one or more times by sulfonic acid.

28. (Amended) [A] The compound[, as claimed in] according to Claim 22, wherein one of said  $R^1$ ,  $R^{1'}$ ,  $R^2$ ,  $R^{2'}$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $[R^7, R^8, R^{15}, R^{16}, R^{20}, R^{21}, R^{23}, R^{24}, R^{25}]$ ,  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ ,  $[R^{37}, R^{38}]$ ,  $R^{41}$ ,  $R^{42}$ ,  $R^{43}$ ,  $R^{44}$ ,  $R^{45}$ , and  $R^{46}$  is [an]  $-L-R_x$  or  $-L-Sc$ .

29. (Amended) [A] The compound[, as claimed in] according to Claim 28, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-2[4]0

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nonhydrogen atoms selected from the group consisting of C, N, O, P, and S<sub>2</sub> [and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds.]

30. (Amended) [A] The compound[, as claimed in] according to Claim 28, wherein said Rx is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, a perfluorobenzamido, an azidoperfluorobenzamido group, [or] and a thiol group.

31. (Amended) [A] The compound[, as claimed in] according to Claim [28] 30, wherein said Rx is independently selected from the group consisting of a phosphoramidite, a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, a perfluorobenzamido, an azidoperfluorobenzamido group, [or] and a reactive platinum complex.

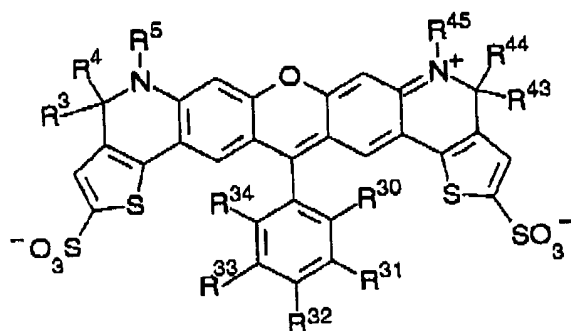
32. (Amended) [A] The compound[, as claimed in] according to Claim 28, wherein said Sc is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a [monosaccharide, a polysaccharide] carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, [or] and a virus.

33. (Amended) [A] The compound[, as claimed in] according to Claim [28] 32, wherein Sc is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, [or] and a nucleic acid.

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34. (Amended) [A] The compound[, as claimed in] according to Claim 28, wherein said compound comprises a [having the] formula:



wherein said  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^{43}$ ,  $R^{44}$ , and  $R^{45}$  are independently methyl or ethyl;

$R^{30}$  is sulfonic acid or carboxylic acid;

$R^{31}$  and  $R^{34}$  are independently H, F, or Cl;

one of  $R^{32}$  and  $R^{33}$  is H, F, or Cl, and the other of  $R^{32}$  and  $R^{33}$  is  $-L-R_x$  or  $-L-S_c$ ,

wherein said L is a covalent linkage [of the formula] comprising -

$S(CH_2)_aCOO(CH_2)_b-$  or [the formula]  $-S(CH_2)_aCONH(CH_2)_b-$

wherein a is an integer between 0 and 10, and b is an integer between 0 and 10

[provided that a and b are not both 0]; and

wherein said  $R_x$  [, where present,] is selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, [or] and a reactive platinum complex.; and

wherein said  $S_c$  [, where present,] is selected from the group consisting of an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a lectin, or a nucleic acid.

35. (Amended) [A] The compound[, as claimed in] according to Claim 34, wherein said  $R_x$  is a maleimide group or is a succinimidyl ester of a carboxylic acid.



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36. (Amended) [A] The compound[, as claimed in] according to Claim 34, wherein said S<sub>c</sub> is a peptide or a protein [or a lectin].

37. (Amended) [A] The compound[, as claimed in] according to Claim 3[4]6, wherein said  $S_c$  is an antibody or antibody fragment or a lectin.

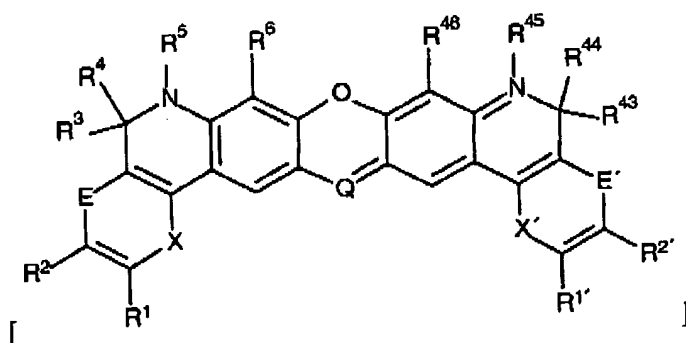
38. (Amended) [A] The compound[, as claimed in] according to Claim 34, wherein said  $S_c$  is a nucleotide or an oligonucleotide.

39. (Amended) [A] The compound[, as claimed in] according to Claim 34, wherein said  $S_c$  is a BAPTA or APTRA ion-complexing moiety.

40. (Amended) A method of staining a [biological] sample, said method comprising steps:

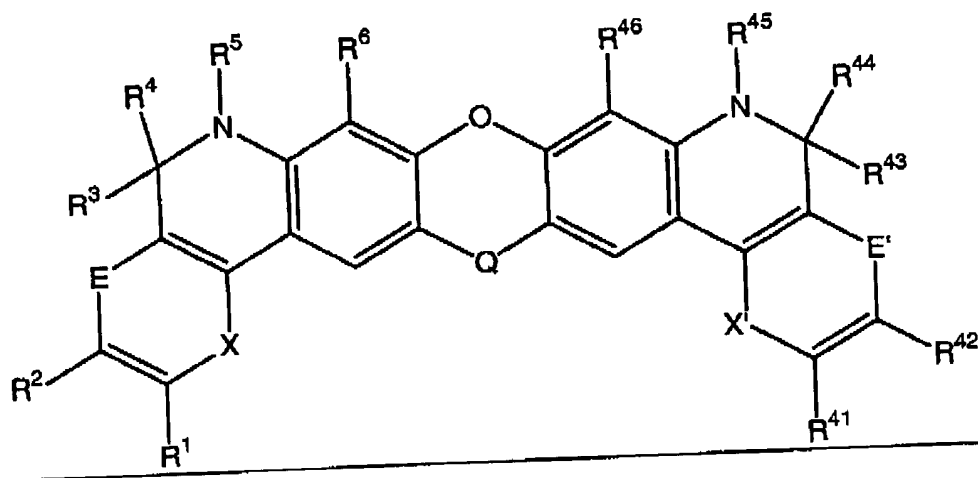
steps:

a) combining a [dye] solution with said sample, wherein said solution comprises [comprising] a compound [of the] having formula



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[wherein]

wherein  $R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$ , and  $R^{46}$  are independently selected from the group consisting of [H] hydrogen, cyano, [nitro,] halogen, carboxylic acid, [or] sulfonic acid; or a)  $C_1$ - $C_6$  alkyl, [or]  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that] and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; [or  $-L-R_x$ ; or  $-L-S_c$ ]

or  $R^1$  in combination with  $R^2$ , or  $R^{41}$  in combination with  $R^{42}$ , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by  $-L-R_x$  or  $-L-S_c$ ;

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently selected from the group consisting of [H] hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or] and said [an] aromatic or heteroaromatic ring [that] is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

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or  $R^2$  in combination with  $R^3$ , or  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [a]  $C_2-C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen[; or  $R^5$  is an] and said aryl or heteroaryl [ring that] is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E [and],  $E'$ ,  $X'$  and X is O, S,  $NR^a$ , or  $CR^{1'}=CR^{2'}$ ; the other is absent; and one of  $E'$  and  $X'$  is O, S,  $NR^a$ , or  $CR^{1'}=CR^{2'}$ ; the other is absent;] provided that E and X or  $E'$  and  $X'$  are not both present;

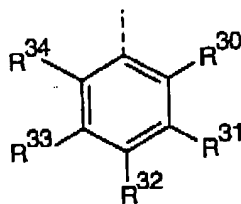
wherein  $R^6$  is independently selected from the group consisting of [H] hydrogen, methyl, carboxymethyl, [or a]  $C_2-C_6$  alkyl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of [H] hydrogen, cyano, halogen, carboxylic acid, [or] sulfonic acid[; or] , [a]  $C_1-C_6$  alkyl, [or]  $C_1-C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy [that] is optionally substituted by carboxylic acid, sulfonic acid, or halogen[; or an aryl or heteroaryl ring] and said aryl or heteroaryl [that] is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

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Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is independently selected from the group consisting of [H] hydrogen, F, CN, carboxylic acid, [or] a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl [that] is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> [has the] comprises a formula



wherein R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently selected from the group consisting of [H] hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or, C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, [or C<sub>6</sub>-C<sub>18</sub>] C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said [the] alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> [which] are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino [or] and C<sub>1</sub>-C<sub>6</sub> alkoxy, the alkyl portions of each having 1-6 carbons]; or [one] a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents [R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>,] when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; [or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>] and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance;

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[with a biological sample in a concentration sufficient to yield a detectable optical response under the desired conditions.]

b) illuminating said sample with a suitable light wavelength to yield a detectable optical response.

41. (Amended) [A] The method [, as claimed in] according to Claim 40, wherein said method further [comprising] comprises combining [the] said sample with an additional detection reagent [that has spectral properties that are detectably different from said optical response].

42. (Cancelled) A method, as claimed in Claim 40, further comprising the step of determining a characteristic of the sample by comparing the optical response with a standard response parameter.

43. (Amended) [A] The method [, as claimed in] according to Claim 40, wherein [the] said sample comprises cells, growth medium, tissue, proteins, peptides, or biological fluids.

44. (Amended) [A] The method [, as claimed in] according to Claim 40, wherein [the] said sample is immobilized in or on a solid or semi-solid matrix that is a membrane, an electrophoretic gel, a silicon chip, a glass slide, a microwell plate, or a microfluidic chip.

45. (Cancelled) A method, as claimed in Claim 40, further comprising tracing the temporal or spatial location of the optical response within the sample.

46. (Amended) [A] The method[, as claimed in] according to Claim 40, wherein [for said compound] at least one of said  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ , and  $R^{34}$ ,  $R^{37}$  and  $R^{38}$ ] is -L- $R_x$  or -L- $Sc$ ;

$R_x$  is selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl

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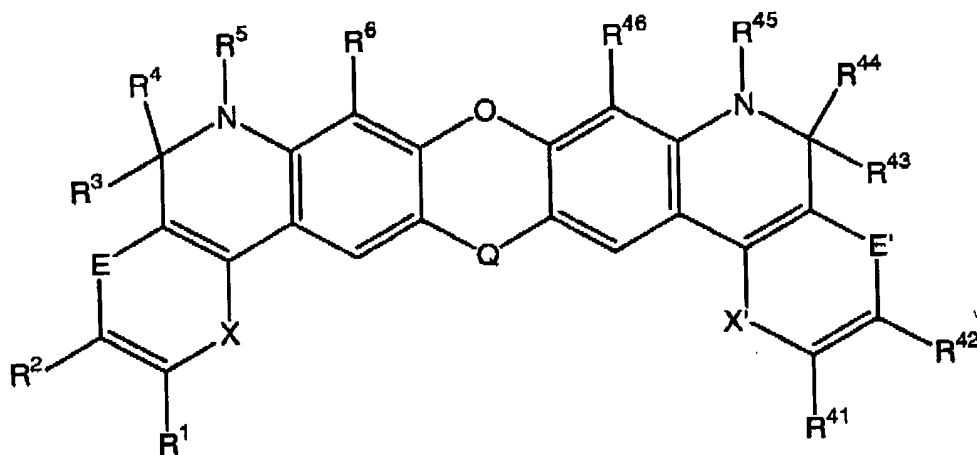
halide, an isothiocyanate, [or] and a maleimide group; and

$S_c$  is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, or a non-biological organic polymer or polymeric microparticle, wherein said  $S_c$  [that] is optionally bound to one or more additional fluorophores [that are the same or different].

47. (Amended) [A] The method[, as claimed in] according to Claim 46, wherein [for said compound,] said  $R^{28}$  is an -L- $S_c$ , and  $S_c$  is an ion-complexing moiety that is a BAPTA or an APTRA.

48. (Amended) [A] The method[, as claimed in] according to Claim 4[0]6, wherein at least one of said  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ , and  $R^{34}$ , [ $R^{37}$  and  $R^{38}$ ] is -L- $S_c$ , and said  $S_c$  is a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid polymer.

49. (New) A kit for staining a sample, wherein said kit comprises  
a solution comprising a buffer and a compound having formula



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wherein  $R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$  and  $R^{46}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^1$  in combination with  $R^2$ , or  $R^{41}$  in combination with  $R^{42}$ , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by  $-L-R_x$  or  $-L-S_c$ ;

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently selected from the group consisting of hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic ring, a heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ , or  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

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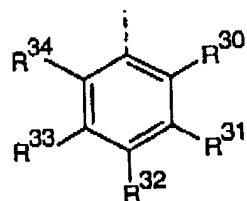
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wherein one of said E, E', X' and X is O, S, NR<sup>8</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>, provided that E and X or E' and X' are not both present;

wherein R<sup>8</sup> is independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C<sub>2</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

R<sup>1'</sup> and R<sup>2'</sup> are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, a C<sub>1</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> comprises a formula



wherein R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazine, C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>38</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkylloxycarbonyl, C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are optionally substituted one or more times by substituents selected from



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the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino and C<sub>1</sub>-C<sub>6</sub> alkoxy; or a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

Sc is a conjugated substance.

50. (New) The kit according to Claim 49, wherein said kit further comprises an additional detection reagent, a purification medium, or standards.

51. (New) The kit according to Claim 49, wherein at least one of said R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>28</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, R<sup>43</sup>, R<sup>44</sup>, R<sup>45</sup> and R<sup>46</sup> is L-R<sub>x</sub> wherein said R<sub>x</sub> is independently selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, and a maleimide group.

52. (New) The kit according to Claim 51, wherein at least one of said R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, or R<sup>34</sup> is L-R<sub>x</sub> and R<sup>30</sup> is carboxylic acid or sulfonic acid.

53. (New) The kit according to Claim 49, wherein at least one of said R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>28</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, R<sup>43</sup>, R<sup>44</sup>, R<sup>45</sup> and R<sup>46</sup> is L-Sc, wherein said Sc is independently selected from the group consisting of an amino acid, a peptide, a protein, an antibody, an antibody fragment, a carbohydrate, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, a non-biological organic polymer and polymeric microparticle.

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54. (New) The kit according to Claim 53, wherein said Sc is an antibody or fragment thereof.

Respectfully submitted,

Date: October 10, 2002

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Diwu *et al.*

Serial No.: 09/922,333

Filed: August 4, 2001

For: **DERIVATIVES OF 1,2-DIHYDRO-7-HYDROXYQUINOLINES CONTAINING FUSED RINGS**

Examiner: F. Powers

Group Art Unit: 1626

**CLEAN VERSION OF THE CLAIMS**

Assistant Commissioner for Patents  
U.S. Patent and Trademark Office  
Washington, D.C. 20231

Dear Sir:

The following Marked-up Version of the Claims is hereby submitted together with a Clean Version of the Claims and the Response to Restriction Requirement on or before the due date of October 10, 2002.

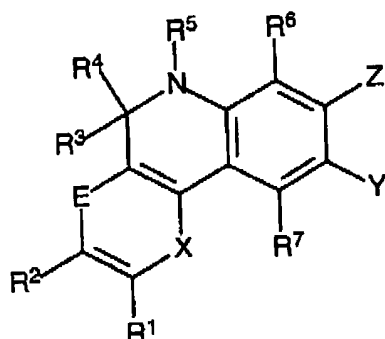
**CERTIFICATE OF TRANSMISSION**

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HEREWITH ARE BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADEMARK OFFICE ON 10/10/02 TO 1.703.872.9306 By [Signature]

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1. (Amended) A compound comprising a formula



wherein

R<sup>1</sup> and R<sup>2</sup> are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R<sub>x</sub> or -L-S<sub>c</sub>;

or R<sup>2</sup> in combination with R<sup>3</sup> forms a 5- or 6-membered alicyclic ring;

R<sup>3</sup> and R<sup>4</sup> are independently selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, aromatic or heteroaromatic ring, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub>

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alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>3</sup> in combination with R<sup>4</sup> forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C<sub>2</sub>-C<sub>6</sub> alkyl, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

R<sup>6</sup> is independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

R<sup>7</sup> is independently selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, -L-R<sub>x</sub> and -L-S<sub>c</sub>;

one of X and E is O, S, NR<sup>a</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>, and the other is absent;

wherein R<sup>a</sup> is independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C<sub>2</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

R<sup>1'</sup> and R<sup>2'</sup> are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl,

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heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

Y is independently selected from the group consisting of H, OH, NH<sub>2</sub>, NO, -(CO)-R<sup>9</sup>, -(CO)-O-R<sup>10</sup>, wherein said R<sup>9</sup> and R<sup>10</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl, or a substituted or unsubstituted aryl or heteroaryl ring system having 1-2 rings;

Z is independently selected from the group consisting of H, OH, NHR<sup>17</sup>, SH, or C(CR<sup>11</sup>R<sup>12</sup>)<sub>2</sub>OH; wherein said R<sup>17</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said R<sup>11</sup> and R<sup>12</sup> are independently C<sub>1</sub>-C<sub>6</sub> alkyl that are optionally substituted by carboxylic acid, sulfonic acid, or halogen, or R<sup>11</sup> and R<sup>12</sup> taken in combination form a 5- or 6-membered alicyclic ring;

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

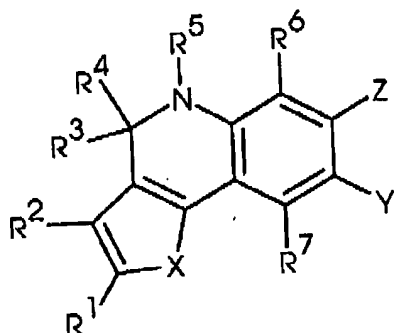
S<sub>c</sub> is a conjugated substance.

2. (Amended) The compound according to Claim 1, wherein one of X and E is O, S, or CR<sup>1'</sup>=CR<sup>2'</sup>, and the other is absent.

3. (Amended) The compound according to Claim 2, wherein said compound has the formula

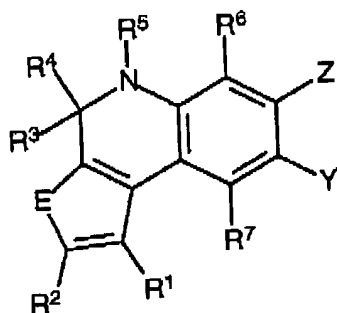
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wherein X is O or S.

4. (Amended) The compound according to Claim 2, wherein said compound has the formula



wherein E is O or S.

5. (Amended) The compound according to Claim 3, wherein X is S.

6. (Amended) The compound according to Claim 1, wherein

R<sup>1</sup> is hydrogen or sulfonic acid;

R<sup>3</sup> and R<sup>4</sup> are each methyl;

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$R^6$  and  $R^7$  are each hydrogen or methyl; and

Z is OH.

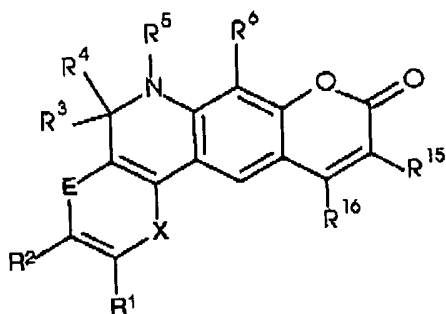
7. (Amended) The compound according to Claim 1, wherein Y is H or  $-(CO)-H$  or  $NO$ .
8. (Amended) The compound according to Claim 1, wherein said L is independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S.
9. (Amended) The compound according to Claim 1, wherein said  $R_x$  is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, and a thiol group.
10. (Amended) The compound according to Claim 1, wherein said  $S_c$  is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, and a virus.



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11. (Amended) A compound comprising a formula



wherein  $R^1$ ,  $R^2$ , and  $R^6$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^1$  in combination with  $R^2$  forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by  $-L-R_x$  or  $-L-S_c$ ;

$R^3$  and  $R^4$  are independently selected from the group consisting of hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ , or  $R^3$  in combination with  $R^4$ , forms a 5- or 6-membered alicyclic ring;

$R^5$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is

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optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

one of X and E is O, S, NR<sup>6</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup> and the other is absent;

wherein R<sup>6</sup> is independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C<sub>2</sub>-C<sub>6</sub> alkyl, -L-R<sub>X</sub> and -L-S<sub>C</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

R<sup>1'</sup> and R<sup>2'</sup> are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>X</sub> and -L-S<sub>C</sub>, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

R<sup>15</sup> and R<sup>16</sup> are independently selected from the group consisting of hydrogen, cyano, nitro, halogen, carboxylic acid, sulfonic acid, C<sub>1</sub>-C<sub>6</sub> alkyl, an aromatic or heteroaromatic ring system having 1-2 fused rings, -L-R<sub>X</sub> and -L-S<sub>C</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aromatic or heteroaromatic ring system is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

wherein L is a covalent linkage;

R<sub>X</sub> is a reactive group; and

S<sub>C</sub> is a conjugated substance.

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12. (Amended) The compound according to Claim 11, wherein said one of X and E is O or S.

13. (Amended) The compound according to Claim 12, wherein

R<sup>6</sup> and R<sup>7</sup> are hydrogen;

R<sup>3</sup> and R<sup>4</sup> are each methyl;

R<sup>1</sup> is hydrogen or sulfonic acid;

one of R<sup>15</sup> and R<sup>16</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>, and the other is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl; or cyano;

wherein L is a single covalent bond, or L is a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S, and

wherein R<sub>x</sub> is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, and a thiol group; and

wherein S<sub>c</sub> is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, and a virus.

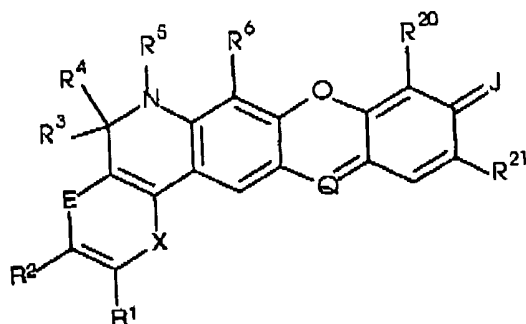
14. (Amended) The compound according to Claim 11, wherein one of said R<sup>15</sup> or R<sup>16</sup> is an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally

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substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl.

15. A compound comprising a formula:



wherein R<sup>1</sup>, R<sup>2</sup>, and R<sup>6</sup> are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R<sub>x</sub> or -L-S<sub>c</sub>;

R<sup>3</sup> and R<sup>4</sup> are independently selected from the group consisting of hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, an aromatic or heteroaromatic ring, L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

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or  $R^2$  in combination with  $R^3$ , or  $R^3$  in combination with  $R^4$ , forms a 5- or 6-membered alicyclic ring;

$R^5$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , forms a 5- or 6-membered alicyclic ring;

one of X and E is O, S,  $NR^6$ , or  $CR^{1'}=CR^{2'}$ , and the other is absent;

wherein  $R^6$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

$R^{20}$  and  $R^{21}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

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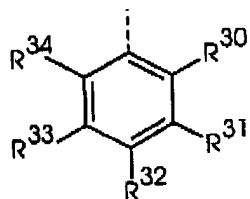
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J is O or  $\text{NR}^{37}\text{R}^{38}$ ;

wherein  $\text{R}^{37}$  and  $\text{R}^{38}$  are independently selected from the group consisting of hydrogen,  $\text{C}_1\text{-C}_6$  alkyl, aryl, heteroaryl,  $-\text{L-R}_x$  and  $-\text{L-Sc}$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $\text{R}^{37}$  in combination with  $\text{R}^{38}$  forms a saturated 5- or 6-membered heterocycle that is a piperidine, a morpholine, a pyrrolidine or a piperazine, wherein said heterocycle is optionally substituted by methyl, carboxylic acid, or a carboxylic acid ester of a  $\text{C}_1\text{-C}_6$  alkyl;

or  $\text{R}^{37}$  in combination with  $\text{R}^{20}$ , or  $\text{R}^{38}$  in combination with  $\text{R}^{21}$ , or both, form a 5- or 6-membered ring that is saturated or unsaturated, and is optionally substituted by one or more sulfonic acids, or  $\text{C}_1\text{-C}_6$  alkyl that is optionally substituted by sulfonic acid;

Q is N or  $\text{CR}^{28}$ , wherein  $\text{R}^{28}$  is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a  $\text{C}_1\text{-C}_6$  alcohol, a  $\text{C}_1\text{-C}_6$  alkyl,  $-\text{L-R}_x$  and  $-\text{L-Sc}$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $\text{R}^{28}$  comprises a formula



wherein  $\text{R}^{30}$ ,  $\text{R}^{31}$ ,  $\text{R}^{32}$ ,  $\text{R}^{33}$  and  $\text{R}^{34}$  are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino,  $\text{C}_1\text{-C}_{18}$  alkyl,  $\text{C}_1\text{-C}_{18}$  alkoxy,  $\text{C}_1\text{-C}_{18}$  alkylthio,  $\text{C}_1\text{-C}_{18}$  alkanoylamino,  $\text{C}_1\text{-C}_{18}$  alkylaminocarbonyl,  $\text{C}_2\text{-C}_{36}$  dialkylaminocarbonyl,  $\text{C}_1\text{-C}_{18}$  alkyloxycarbonyl,  $\text{C}_7\text{-C}_{18}$  arylcarboxamido,  $-\text{L-R}_x$  and  $-\text{L-Sc}$ , wherein said alkyl or aryl portions of said  $\text{R}^{30}$ ,  $\text{R}^{31}$ ,  $\text{R}^{32}$ ,  $\text{R}^{33}$  and  $\text{R}^{34}$  are optionally substituted one or more times by substituents selected from

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the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino and C<sub>1</sub>-C<sub>6</sub> alkoxy; or a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance.

16. (Amended) The compound according to Claim 15, wherein said Q is N.

17. (Amended) The compound according to Claim 15, wherein said J is O and said Q is CR<sup>28</sup>.

18. (Amended) The compound according to Claim 17, wherein one of said R<sup>5</sup>, R<sup>21</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>.

19. (Amended) The compound according to Claim 15, wherein

said R<sup>3</sup> and R<sup>4</sup> are each methyl;

R<sup>1</sup> is H or a sulfonic acid;

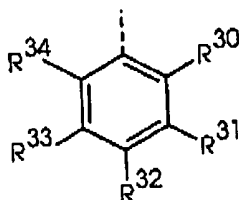
R<sup>6</sup> is H; and

J is NR<sup>37</sup>R<sup>38</sup>.

20. (Amended) The compound according to Claim 19, wherein Q is CR<sup>28</sup> and R<sup>28</sup> has the formula

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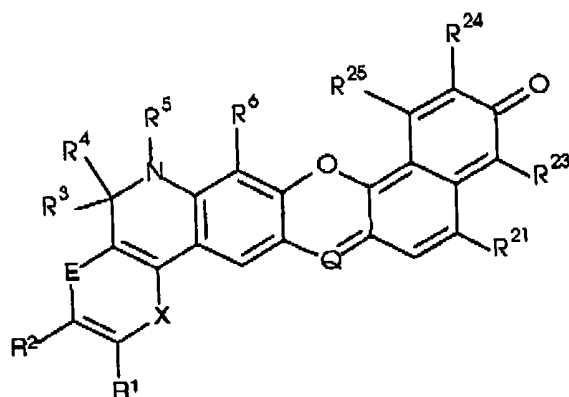
wherein one of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>; and  
wherein L is a single covalent bond, or L is a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S, and  
wherein R<sub>x</sub> is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, and a thiol group; and  
wherein S<sub>c</sub> is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, and a virus.

21. (Amended) A compound comprising a formula



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wherein  $R^1$ ,  $R^2$ , and  $R^6$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^1$  in combination with  $R^2$  forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by  $-L-R_x$  or  $-L-S_c$ ;

$R^3$  and  $R^4$  are independently selected from the group consisting of hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ , or  $R^3$  in combination with  $R^4$ , forms a 5- or 6-membered alicyclic ring;

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$R^5$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2-C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , forms a 5- or 6-membered alicyclic ring;

one of E and X is O, S,  $NR^a$ , or  $CR^{1'}=CR^{2'}$ , and the other is absent;

wherein  $R^6$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2-C_6$  alkyl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid,  $C_1-C_6$  alkyl,  $C_1-C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

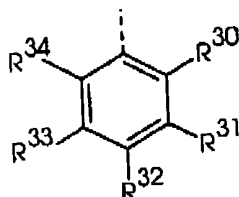
$R^{21}$ ,  $R^{23}$ ,  $R^{24}$ , and  $R^{25}$  are independently selected from the group consisting of hydrogen, cyano, nitro, halogen, carboxylic acid, sulfonic acid,  $C_1-C_6$  alkyl, aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, or halogen said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

Q is N or  $CR^{28}$ , wherein  $R^{28}$  is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a  $C_1-C_6$  alcohol, a  $C_1-C_6$  alkyl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid,

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sulfonic acid, amino, or halogen; or  $R^{28}$  comprises a formula



wherein  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino,  $C_1$ - $C_{18}$  alkyl,  $C_1$ - $C_{18}$  alkoxy,  $C_1$ - $C_{18}$  alkylthio,  $C_1$ - $C_{18}$  alkanoylamino,  $C_1$ - $C_{18}$  alkylaminocarbonyl,  $C_2$ - $C_{36}$  dialkylaminocarbonyl,  $C_1$ - $C_{18}$  alkyloxycarbonyl,  $C_7$ - $C_{18}$  arylcarboxamido,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or aryl portions of said  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a  $C_1$ - $C_6$  alcohol, sulfonic acid, amino,  $C_1$ - $C_6$  alkylamino,  $C_2$ - $C_6$  dialkylamino and  $C_1$ - $C_6$  alkoxy; or a pair of adjacent  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

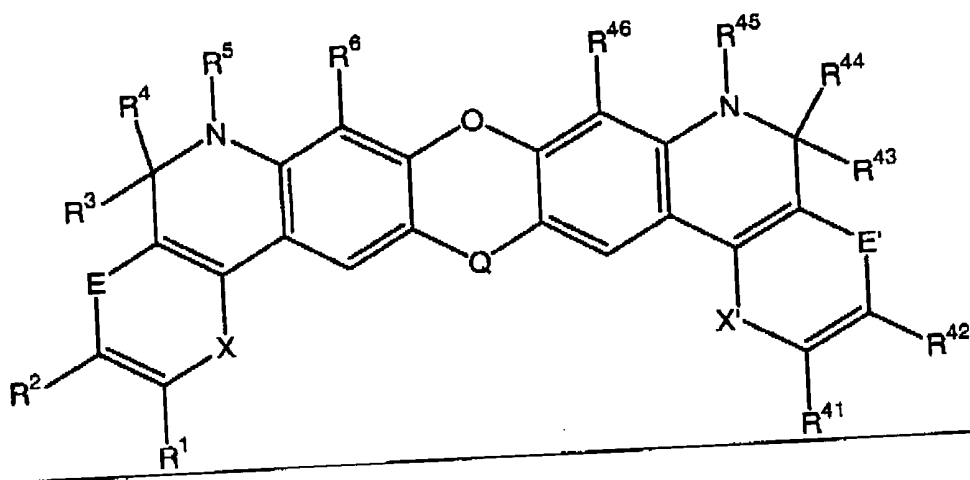
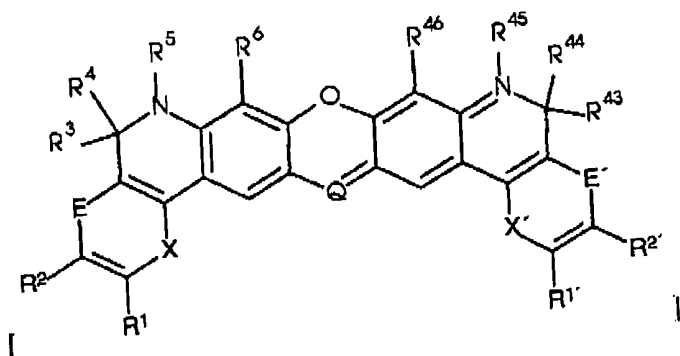
$R_x$  is a reactive group; and

$S_c$  is a conjugated substance.

22. (Amended) A compound comprising a formula:

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wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>6</sup>, R<sup>41</sup>, R<sup>42</sup>, and R<sup>46</sup> are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, aryl, heteroaryl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>1</sup> in combination with R<sup>2</sup>, or R<sup>41</sup> in combination with R<sup>42</sup>, or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R<sub>x</sub> or -L-S<sub>c</sub>;

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$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently selected from the group consisting of hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ , or  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E, E', X' and X is O, S,  $NR^8$ , or  $CR^{1'}=CR^{2'}$  provided that E and X or E' and X' are not both present;

wherein  $R^8$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

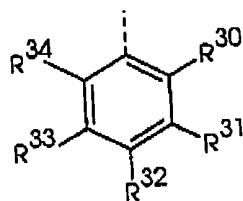
$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is

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optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, a C<sub>1</sub>-C<sub>6</sub> alkyl, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> comprises a formula



wherein R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino, C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino and C<sub>1</sub>-C<sub>6</sub> alkoxy; or a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance.

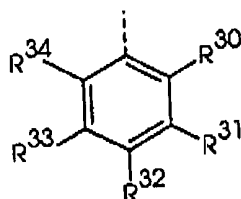
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23. (Cancel) A compound, as claimed in Claim 22, wherein

$X = X'$ ,  $E = E'$ ,  $R^1 = R^{41}$ , and  $R^2 = R^{42}$ .

24. (Amended) The compound according to Claim 22, wherein Q is  $CR^{28}$  and  $R^{28}$  has the formula



25. (Amended) The compound according to Claim 24, wherein one of  $R^5$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ , and  $R^{45}$  is  $-L-R_x$  or  $-L-S_c$ .

26. (Amended) The compound according to Claim 24, wherein

said  $R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are each methyl;

each  $R^1$  and  $R^{41}$  is independently H or sulfonic acid; and

$R^6$  and  $R^{46}$  are H.

27. (Amended) The compound according to Claim 24, wherein said compound is substituted one or more times by sulfonic acid.

28. (Amended) The compound according to Claim 22, wherein one of said  $R^1$ ,  $R^{1'}$ ,  $R^2$ ,  $R^{2'}$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ ,  $R^{41}$ ,  $R^{42}$ ,  $R^{43}$ ,  $R^{44}$ ,  $R^{45}$ , and  $R^{46}$  is  $-L-R_x$  or  $-L-S_c$ .

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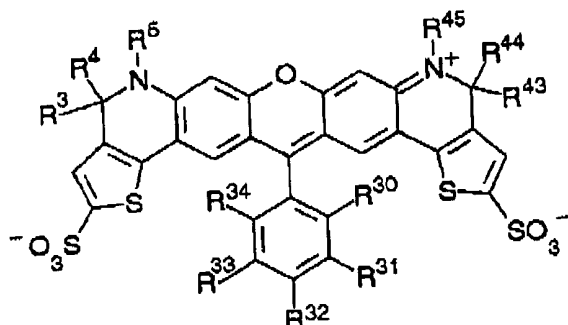
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29. (Amended) The compound according to Claim 28, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S.
30. (Amended) The compound according to Claim 28, wherein said R<sub>x</sub> is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, a perfluorobenzamido, an azidoperfluorobenzamido group, and a thiol group.
31. (Amended) The compound according to Claim 30, wherein said R<sub>x</sub> is independently selected from the group consisting of a phosphoramidite, a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, a perfluorobenzamido, an azidoperfluorobenzamido group, and a reactive platinum complex.
32. (Amended) The compound according to Claim 28, wherein said S<sub>c</sub> is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, and a virus.
33. (Amended) The compound according to Claim 32, wherein S<sub>c</sub> is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, and a nucleic acid.
34. (Amended) The compound according to Claim 28, wherein said compound comprises a formula:



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wherein said  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^{43}$ ,  $R^{44}$ , and  $R^{45}$  are independently methyl or ethyl;

$R^{30}$  is sulfonic acid or carboxylic acid;

$R^{31}$  and  $R^{34}$  are independently H, F, or Cl;

one of  $R^{32}$  and  $R^{33}$  is H, F, or Cl, and the other of  $R^{32}$  and  $R^{33}$  is  $-L-R_x$  or  $-L-S_c$ ,

wherein said L is a covalent linkage comprising  $-S(CH_2)_aCOO(CH_2)_b-$  or  $-S(CH_2)_aCONH(CH_2)_b-$

wherein a is an integer between 0 and 10, and b is an integer between 0 and 10;  
 and

wherein said  $R_x$  is selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, and a reactive platinum complex.; and

wherein said  $S_c$  is selected from the group consisting of an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a lectin, or a nucleic acid.

35. (Amended) The compound according to Claim 34, wherein said  $R_x$  is a maleimide group or is a succinimidyl ester of a carboxylic acid.

36. (Amended) The compound according to Claim 34, wherein said  $S_c$  is a peptide or a protein.

37. (Amended) The compound according to Claim 36, wherein said  $S_c$  is an antibody or

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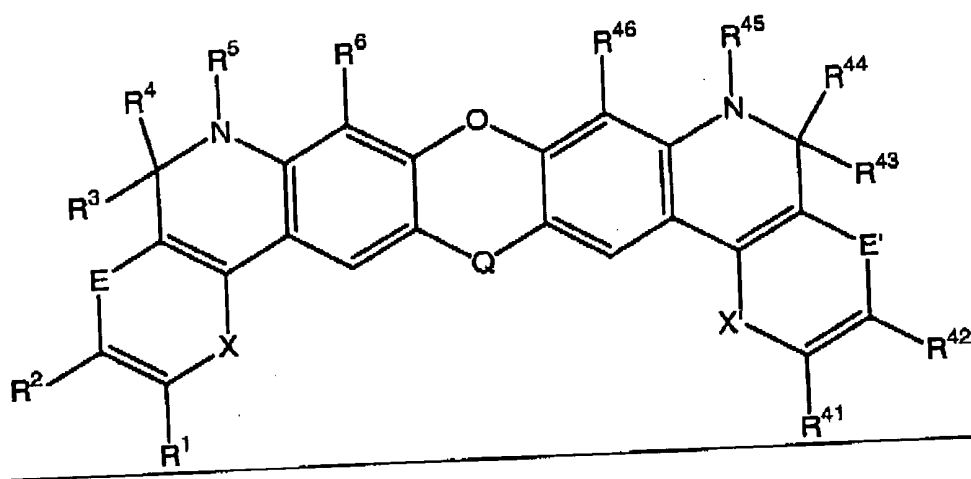
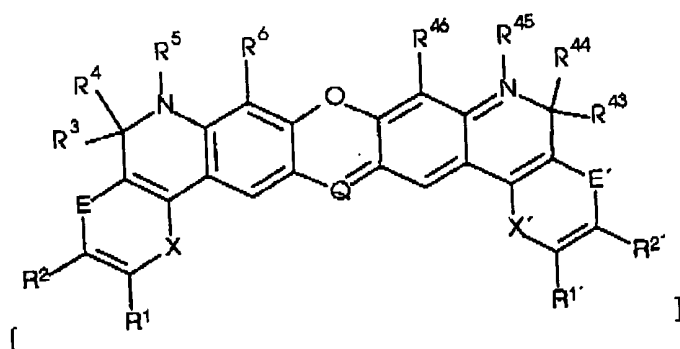
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antibody fragment or a lectin.

38. (Amended) The compound according to Claim 34, wherein said  $S_c$  is a nucleotide or an oligonucleotide.

39. (Amended) The compound according to Claim 34, wherein said  $S_c$  is a BAPTA or APTRA ion-complexing moiety.

40. (Amended) A method of staining a sample, said method comprising steps:  
a) combining a solution with said sample, wherein said solution comprises a compound having formula



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wherein  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^{41}$ ,  $R^{42}$ , and  $R^{46}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^1$  in combination with  $R^2$ , or  $R^{41}$  in combination with  $R^{42}$ , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by  $-L-R_x$  or  $-L-S_c$ ;

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently selected from the group consisting of hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic or heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^2$  in combination with  $R^3$ , or  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

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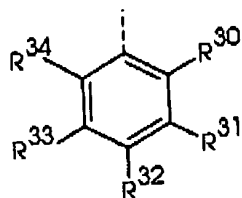
or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E, E', X' and X is O, S,  $NR^a$ , or  $CR^{1'}=CR^{2'}$  provided that E and X or E' and X' are not both present;

wherein  $R^a$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2-C_6$  alkyl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid,  $C_1-C_6$  alkyl,  $C_1-C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1-C_6$  alkyl,  $C_1-C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

Q is N or  $CR^{2b}$ , wherein  $R^{2b}$  is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a  $C_1-C_6$  alcohol, a  $C_1-C_6$  alkyl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^{2b}$  comprises a formula



wherein  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$  and  $R^{34}$  are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino,  $C_1-C_{18}$  alkyl,  $C_1-C_{18}$  alkoxy,  $C_1-C_{18}$  alkylthio,  $C_1-C_{18}$  alkanoylamino,  $C_1-C_{18}$

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alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino and C<sub>1</sub>-C<sub>6</sub> alkoxy; or a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance;

b) illuminating said sample with a suitable light wavelength to yield a detectable optical response.

41. (Amended) The method according to Claim 40, wherein said method further comprises combining said sample with an additional detection reagent.

42. (Cancelled) A method, as claimed in Claim 40, further comprising the step of determining a characteristic of the sample by comparing the optical response with a standard response parameter.

43. (Amended) The method according to Claim 40, wherein said sample comprises cells, growth medium, tissue, proteins, peptides, or biological fluids.

44. (Amended) The method according to Claim 40, wherein said sample is immobilized in or on a solid or semi-solid matrix that is a membrane, an electrophoretic gel, a silicon chip, a glass slide, a microwell plate, or a microfluidic chip.

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45. (Cancelled) A method, as claimed in Claim 40, further comprising tracing the temporal or spatial location of the optical response within the sample.
46. (Amended) The method according to Claim 40, wherein at least one of said  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ , and  $R^{34}$  is -L- $R_x$  or -L- $S_c$ ;

$R_x$  is selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, and a maleimide group; and

$S_c$  is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, or a non-biological organic polymer or polymeric microparticle, wherein said  $S_c$  is optionally bound to one or more additional fluorophores.

47. (Amended) The method according to Claim 46, wherein said  $R^{28}$  is an -L- $S_c$ , and  $S_c$  is an ion-complexing moiety that is a BAPTA or an APTRA.

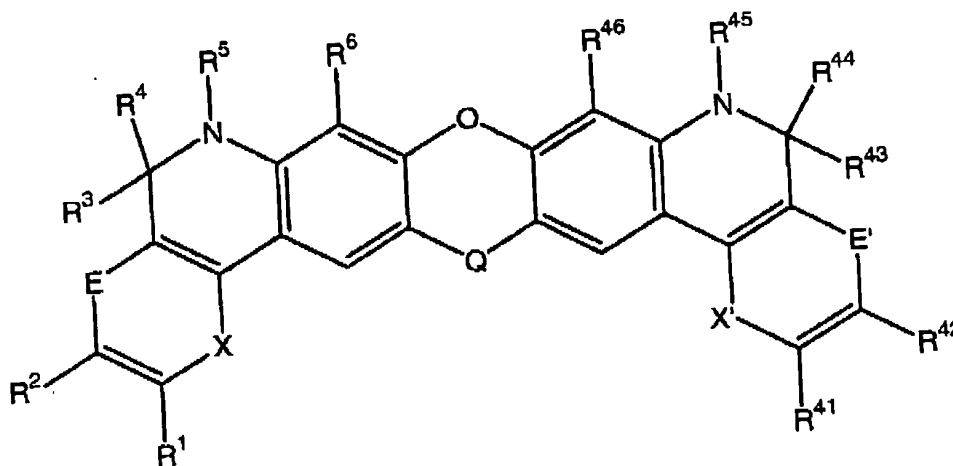
48. (Amended) The method according to Claim 46, wherein at least one of said  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ , and  $R^{34}$  is -L- $S_c$ , and said  $S_c$  is a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid polymer.

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49. (New) A kit for staining a sample, wherein said kit comprises a solution comprising a buffer and a compound having formula

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wherein  $R^1$ ,  $R^2$ ,  $R^6$ ,  $R^{41}$ ,  $R^{42}$  and  $R^{46}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^1$  in combination with  $R^2$ , or  $R^{41}$  in combination with  $R^{42}$ , or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by  $-L-R_x$  or  $-L-S_c$ ;

$R^3$ ,  $R^4$ ,  $R^{43}$ , and  $R^{44}$  are independently selected from the group consisting of hydrogen,  $C_1$ - $C_6$  alkyl, an aromatic ring, a heteroaromatic ring,  $-L-R_x$  and  $-L-S_c$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

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or  $R^2$  in combination with  $R^3$ , or  $R^{42}$  in combination with  $R^{43}$ , or  $R^3$  in combination with  $R^4$ , or  $R^{43}$  in combination with  $R^{44}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

$R^5$  and  $R^{45}$  are independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl, aryl, heteroaryl,  $-L-R_x$  and  $-L-Sc$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or  $R^4$  in combination with  $R^5$ , or  $R^5$  in combination with  $R^6$ , or  $R^{44}$  in combination with  $R^{45}$ , or  $R^{45}$  in combination with  $R^{46}$ , or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E, E', X' and X is O, S,  $NR^a$ , or  $CR^{1'}=CR^{2'}$ , provided that E and X or E' and X' are not both present;

wherein  $R^8$  is independently selected from the group consisting of hydrogen, methyl, carboxymethyl,  $C_2$ - $C_6$  alkyl,  $-L-R_x$  and  $-L-Sc$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

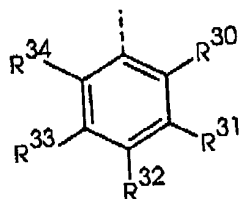
$R^{1'}$  and  $R^{2'}$  are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid,  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  alkoxy, aryl, heteroaryl,  $-L-R_x$  and  $-L-Sc$ , wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by  $C_1$ - $C_6$  alkyl,  $C_1$ - $C_6$  perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

Q is N or  $CR^{28}$ , wherein  $R^{28}$  is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a  $C_1$ - $C_6$  alcohol, a  $C_1$ - $C_6$  alkyl,  $-L-R_x$  and  $-L-Sc$ , wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or  $R^{28}$  comprises a formula



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wherein R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazine, C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, C<sub>7</sub>-C<sub>18</sub> arylcarboxamido, -L-R<sub>x</sub> and -L-S<sub>c</sub>, wherein said alkyl or aryl portions of said R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, C<sub>1</sub>-C<sub>6</sub> alkylamino, C<sub>2</sub>-C<sub>6</sub> dialkylamino and C<sub>1</sub>-C<sub>6</sub> alkoxy; or a pair of adjacent R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance.

50. (New) The kit according to Claim 49, wherein said kit further comprises an additional detection reagent, a purification medium, or standards.

51. (New) The kit according to Claim 49, wherein at least one of said R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>28</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, R<sup>43</sup>, R<sup>44</sup>, R<sup>45</sup> and R<sup>46</sup> is L-R<sub>x</sub> wherein said R<sub>x</sub> is independently selected from the group consisting of a carboxylic acid, an activated ester

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of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, and a maleimide group.

52. (New) The kit according to Claim 51, wherein at least one of said  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ , or  $R^{34}$  is  $L-R_x$  and  $R^{30}$  is carboxylic acid or sulfonic acid.

53. (New) The kit according to Claim 49, wherein at least one of said  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^{28}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ ,  $R^{33}$ ,  $R^{34}$ ,  $R^{43}$ ,  $R^{44}$ ,  $R^{45}$  and  $R^{46}$  is  $L-Sc$ , wherein said  $Sc$  is independently selected from the group consisting of an amino acid, a peptide, a protein, an antibody, an antibody fragment, a carbohydrate, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, a non-biological organic polymer and polymeric microparticle.

54. (New) The kit according to Claim 53, wherein said  $Sc$  is an antibody or fragment thereof.

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Respectfully submitted,

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